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Induction of Physical Dependence Upon Ethanol in Rats Using Intravenous Infusion[1]

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NUMAN, R. AND A. M. GILROY. *Induction of physical dependence upon ethanol in rats using intravenous infusion.* PHARMAC. BIOCHEM. BEHAV. 9(3) 279–282, 1978.—Intravenous infusions were used to produce physical dependence upon ethanol in rats. The procedure proved to be safe, rapid, and reliable. Ethanol (30% v/v) was administered over a 7-day period. The mean daily dose ranged from 10–14 g/kg/day. Control rats were exposed to a comparable procedure except that saline, rather than ethanol, was infused. All ethanol treated rats that survived the intoxication period (n=11) showed signs of physical dependence (moderate to severe, n=8; mild, n=3) following ethanol withdrawal. Saline treated rats (n=8) did not show any of these symptoms. The most reliable ethanol withdrawal signs observed were: spontaneous seizure (n=7), audiogenic seizure (n=7), tremors (n=6), tail stiffening (n=10) and body rigidity (n=9). These symptoms were analyzed in terms of their hour of onset and hour of maximum intensity following ethanol withdrawal. Application of the intravenous method for the study of ethanol self-administration is discussed.

Ethanol physical dependence Intravenous infusion Alcohol withdrawal syndrome Rats

DURING the last decade a number of investigators have attempted to develop animal models of alcoholism [5, 6, 15, 16]. While several criteria have been established for such a model [6,12], two of these criteria have received the greatest research attention. First, during withdrawal from alcohol, specific neurological signs of abstinence, indicative of physical dependence, should develop. Second, the experimental animal should voluntarily self-administer intoxicating quantities of alcohol.

Several investigators have recently achieved the first criterion using pharmacological (forced administration of ethanol) models [16]. Forced administration procedures must be used because most laboratory animals will not voluntarily consume ethanol solutions in quantities sufficient to cause physical dependence [6,17]. The most successful pharmacological models have included intragastric [3, 13, 14], liquid diet [4, 10, 20], and inhalation [9,21] procedures for the administration of ethanol. These procedures have been employed in dependence studies using rats [10, 13, 14, 21], mice [4,9], dogs [3] and monkeys [20]. However, with the exception of some recent work using intragastric methods [2], these procedures have not been successfully employed for the production of voluntary self-administration of intoxicating quantities of ethanol.

The intravenous (IV) procedure has not been a popular candidate for animal studies on alcohol dependence. While a few investigators [1,16] have used the IV procedure in monkeys, other, less expensive species have not been tested for ethanol dependence or withdrawal induced self-administration of ethanol using this method. However, recent investigations have successfully applied the IV method in rats to assess the euphoric effects of ethanol [23,25]. Considering these recent advances, and the success achieved with the IV method for developing animal models of opiate dependence [7, 8, 18, 19, 22, 27], we attempted to utilize this procedure for the reliable induction of physical dependence upon ethanol in rats.

METHOD

Animals and Apparatus

Male hooded rats of the Long-Evans strain which weighed between 240–340 g were used. Each rat was implanted with an indwelling jugular cannula while under Nembutal anesthesia (50 mg/kg). The cannula was passed from the jugular vein, subcutaneously, to exit at the dorsal region of the animal's neck. The rat was then placed in a harness which had a spring (30 cm in length) attached to it, and the cannula was passed through this protective spring. Each rat was then individually housed in a sound attenuated behavioral chamber. The spring was clamped to the top of the animal's cage, and the cannula tubing was passed to an injection system located outside the chamber (Harvard Apparatus Compact Syringe Pump, model 975). More detailed descriptions of the surgical procedure, and directions

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for cannula and harness construction can be found in recent publications [24,26].

The animals remained in the behavioral chambers 24 hr a day throughout the entire experiment. Food (granulated) and water (in calibrated drinking tubes) were freely available at all times. The chambers were well ventilated, temperature controlled (23 ± 1°C), and internal lighting alternated on a 12 hr day-night (0800–2000 hr) cycle. The scheduling of saline and ethanol infusions was automatically programmed with electromechanical circuitry.

Procedure

During the first 3 postoperative days the rats received 1 IV infusion of sterile saline (3 ml at a rate of 0.3 ml/min) every 4 hr around the clock. These 3 saline days served as an habituation and postoperative recovery period, and allowed food and water intake to stabilize.

Following this habituation period, ethanol infusions were initiated in experimental rats (n=17) and saline infusions were continued in control animals (n=8). The concentration of ethanol used was 30% v/v (prepared from 95% ethanol and sterile saline). For the experimental animals, ethanol was administered over a 7-day period (interanimal range 5–10 days). The mean daily dose ranged from 10–14 g/kg/day. This dose was administered in 4–5 fractional doses over each 24 hr period at a rate of 0.3 ml/min. The mean duration of each injection ranged between 8.9 and 12.4 min. A comparable procedure was used for control animals with the exception that saline, rather than ethanol, was infused. All rats were checked, several times each day, for gross signs of intoxication (ataxia, loss of eyeblink and righting reflexes, coma).

It should be pointed out that available equipment only allowed the testing of 4 animals at any one time. Therefore, the 25 rats were usually tested in batches of 3 or 4 rats at approximately 3-week intervals. Each batch of rats consisted of at least 1 control animal.

Following the intoxication phase, the withdrawal phase was initiated. Ethanol (or saline in the case of controls) infusions were usually discontinued at approximately 0800 hr. The rats were then observed hourly between 1 and 18 hr after withdrawal and thereafter at approximately 6 hr intervals (up to 72 hr following withdrawal) for signs of physical dependence. For these observations, the door to the behavioral chamber was opened, but the animal was not removed. Each rat was observed for approximately 5 min. The occurrence of withdrawal signs was recorded, and each withdrawal symptom was rated on a scale of 0 (absent) to 3 (severe) according to its intensity. For the characterization and grading of these abstinence signs we relied heavily on the reports of Majchrowicz [13] and Irwin [11]. At the end of each 5 min observation period the observer jangled a bunch of keys in front of the rat in an attempt to induce audiogenic seizure activity.

Food and water consumption were measured daily throughout the experiment. Body weights were measured daily during the habituation and intoxication phases, but not during the withdrawal phase.

RESULTS

Mortality

Of the 17 rats exposed to ethanol, 6 (35%) died during the intoxication phase. However, most of these died during our early attempts at applying the IV procedure. Of the last 8 animals attempted, only 1 rat died during intoxication. We suggest that the dosing guidelines outlined by Majchrowicz [13] be followed when employing the IV procedure for the induction of physical dependence on ethanol in rats. Two additional rats died as a result of withdrawal seizures. One died during a spontaneous seizure, and the other during an induced (audiogenic) seizure.

Characterization of the Intoxication Phase

All ethanol treated rats remained intoxicated throughout the entire infusion period. However, the degree of intoxication, as indicated by behavioral correlates [13], varied from animal to animal. Immediately following an ethanol infusion most rats showed a loss of the righting reflex and some animals became comatose. Most animals regained the righting reflex within 2 hr following an ethanol infusion, but remained mildly ataxic and sedated for the remainder of the interinfusion interval (there was some development of tolerance to these effects, but we did not systematically measure these changes). Occasionally a rat remained severely intoxicated (loss of righting and eyeblink reflexes) throughout the entire interinfusion interval. In such cases the next scheduled ethanol infusion was skipped in order to allow the rat to recover, and to prevent death [13].

All rats receiving ethanol infusions showed a dramatic reduction in food (78%) and water (54%) intake by the end of the intoxication phase, and a concomitant reduction in body weight (9%). These data are shown in Table 1. In contrast, saline treated control rats remained stable or showed an increase on these parameters (data not shown). These data are in agreement with previous findings using intragastric methods [13].

TABLE 1

CHANGES IN FOOD AND WATER CONSUMPTION, AND BODY WEIGHT DURING THE ETHANOL INTOXICATION PHASE

	Mean ± Standard Error		
	Food g/Day	Water ml/Day	Body Weight (g)
Last 2 Days Saline	22.5 ± 1.2	30.6 ± 2.4	312.8 ± 10.8
Last 2 Days Ethanol	5.0 ± 2.0	14.1 ± 3.0	285.9 ± 10.9
Change	-17.5 ± 2.6	-16.5 ± 4.4	-26.9 ± 5.3
P*	<0.01	<0.01	<0.001
N	11	11	11

*Dependent t-test, two tailed

Characterization of the Withdrawal Phase

All ethanol treated rats that survived the intoxication phase (n=11) showed withdrawal signs. Saline treated controls did not show any of these symptoms. The most reliable ethanol withdrawal signs were spontaneous seizures (SS), audiogenic seizures (AS), tremors (T), tail stiffening (TS) and body rigidity (R). TS was usually accompanied by tail elevation (horizontal, diagonal and vertical were often observed). Other symptoms (body and head shakes, head flicks and head search, stereotypy, irritability, teeth chattering, lacrimation and salivation) were also observed, but were less reliable. Therefore, we used only SS, AS, T, TS, and R in rating the severity of the ethanol withdrawal syndrome.

Table 2 shows, for each rat, the maximum intensity rating achieved for each withdrawal sign during the first 18 hr after withdrawal. As can be seen, 64% of the rats showed SS and

TABLE 2

MAXIMUM INTENSITY OF ETHANOL WITHDRAWAL SIGNS

Rat	Maximum Intensity Rating For Each Withdrawal Sign*						Overall Severity Score†	Withdrawal Classification†	Days of Exposure to Ethanol
	SS	AS	T	TS	R	Death			
1	2	1	2	2	2	—	9	Moderate	10
2	2	1	2	2	3	—	10	Moderate	10
3	0	0	0	2	0	—	2	Mild	7
4	0	1	0	0	2	—	3	Mild	7
5	2	3	2	3	3	Yes	13	Severe	8
6	0	2	2	2	3	—	9	Moderate	6.5
7	0	2	0	2	0	—	4	Mild	6.5
8	3	3	0	3	2	Yes	11	Severe	6.5
9	2	0	3	3	3	—	11	Severe	6.5
10	2	0	0	3	3	—	8	Moderate	5
11	2	0	2	3	3	—	10	Moderate	5
N‡	7	7	6	10	9	2			

*Each score indicates maximum observed intensity rating (0–3) for each symptom during the first 18 hr after withdrawal.
†See text for explanation.
‡Number of rats showing symptom.

AS, 55% showed T, 91% showed TS and 82% showed R. Based on the constellation of symptoms observed, each rat was assigned an overall severity score. While many procedures can be used to arrive at an overall severity score [9], we simply summated the individual intensity ratings for each withdrawal sign. Using this method, the maximum score possible is 15 (3×5). Our ethanol treated rats achieved a mean severity score of 8.2, with a standard error of 1.1. Further, we classified severity scores between 1 and 5 as indicative of mild withdrawal, between 6 and 10 as moderate withdrawal, and above 10 as severe withdrawal. Based on this analysis, 3 rats were classified as mild, 3 as severe, and 5 as moderate. Table 2 also shows that there was not a clear relationship between length of exposure to ethanol and withdrawal severity. Five days of exposure to ethanol were sufficient to produce good withdrawal effects.

There was a small positive correlation between withdrawal severity scores and body weight loss (mean weight loss during the last 2 days of the intoxication period). However, this correlation was not significant (Spearman Rank Correlation, $r_s=0.32$, $p>0.1$). Therefore, it seems unlikely that body weight loss contributed to withdrawal severity.

Table 3 shows, for each symptom, the mean hr of first occurrence, and the mean hr of peak intensity onset (first occurrence of maximum intensity rating). These means were calculated only on data for rats which showed a particular symptom. At the bottom of Table 3 symptoms have been classified further into 2 categories. Tonic symptoms include TS and R because they occurred continuously following initial onset. Phasic symptoms include SS, AS, and T since these symptoms occurred only intermittently. For both classifications, however, symptom intensity varies over time. Table 3 shows that withdrawal symptoms appear in an orderly temporal sequence (R<TS<T=AS<SS). In contrast, the onset of peak intensity was expressed at about the same time following ethanol withdrawal for all symptoms (between 10 and 12 hr for all symptoms except SS which peaked out at 14 hr). These impressions are clearer when we compare tonic with phasic symptoms. The onset of tonic symptoms occurred significantly earlier during the withdrawal period when compared to phasic symptoms (t-test, $p<0.01$). However, the time of symptom peak intensity did not differ for tonic and phasic symptoms (t-test, $p>0.10$).

Lastly, most symptoms began to decrease in intensity between 18 and 24 hr after withdrawal, and most symptoms were greatly reduced by 30 hr. However, TS and R could be observed as late as 72 hr after withdrawal in some rats.

TABLE 3

TIME OF FIRST OCCURRENCE AND PEAK INTENSITY ONSET FOR ETHANOL WITHDRAWAL SIGNS

Symptom	N	Hour of Onset After Withdrawal (Mean ± SE)	
		First Occurrence	Peak Intensity
SS	7	13.6 ± 0.87	14.0 ± 1.11
AS	7	10.7 ± 1.50	11.1 ± 1.53
T	6	10.2 ± 0.91	11.5 ± 1.12
TS	10	7.3 ± 0.45	10.7 ± 0.50
R	9	4.8 ± 0.70	12.0 ± 0.96
Phasic*	10	11.3 ± 0.89	11.9 ± 0.98
Tonic*	11	6.1 ± 0.67	11.2 ± 0.54

*The means (±SE) for Phasic (SS, AS,T) and Tonic (TS, R) symptoms were derived by assigning average hour scores to each rat. These average scores were based only on the symptoms that each rat showed within each classification (Phasic and Tonic). Since most rats did not show all symptoms within each classification there is some loss of information with this procedure. These average hour scores were then summed across rats to obtain the means shown. One rat did not show any phasic symptoms and was therefore not included in the calculation of 'phasic' means.

DISCUSSION

The current investigation has shown that the intravenous procedure can be safely used (we did not observe indications of thrombophlebitis or cannula blockage) to reliably produce physical dependence on ethanol in rats. The intravenous procedure has a number of advantages compared to other methods. The IV method allows automated and precise control of alcohol infusion rate and duration of exposure. Further, the IV method is easily adapted to studies of self-administration behavior employing automated operant paradigms [18, 22–27] and may overcome gustatory and olfactory aversions to ethanol. Therefore, the application of the IV method in rats may open a new avenue for the careful assessment of factors involved in the development of voluntary ethanol self-selection [23,25] and self-intoxication. In addition to these advantages, the IV procedure proved to be rapid (5 days of intoxication proved to be sufficient to induce physical dependence) and adjunctive procedures such as weight reduction [15] and pyrazole injections [9] were not necessary.

The characterization of ethanol withdrawal symptoms using the IV procedure was similar to that described for rodents using other procedures [4, 10, 13, 14]. In general, dependent animals begin showing mild signs of physical dependence at about 6 hr after withdrawal. These symptoms are of a tonic character in that they can be observed continuously throughout the withdrawal period. As time since the last ethanol infusion increases, the intensity of these tonic symptoms (R, TS) also increases. When tonic symptoms reach their maximum intensity (about 11 hr after withdrawal) phasic symptoms (SS, AS, T) begin to occur. At time of onset, the phasic symptoms are usually expressed near maximum intensity, and can be observed intermittently up to at least 18 hr after withdrawal. Reduction in symptom intensity begins at about 18 hr after withdrawal, and is greatly reduced by 30 hr. Symptoms tended to disappear in the opposite order of their occurrence, with phasic symptoms disappearing first, followed by the tonic symptoms.

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